

(FILE 'CAPLUS' ENTERED AT 14:38:59 ON 03 DEC 2003)

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L1 346 S (PAPILLOMAVIRUS) AND (DNA (2W)BIND?)
L2 11 S L1 AND NMR

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L2 ANSWER 1 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2003:267305 CAPLUS
DN 139:2621
TI Domain Substructure of HPV E6 Oncoprotein: Biophysical Characterization of the E6 C-Terminal **DNA-Binding** Domain
AU Nomine, Yves; Charbonnier, Sebastian; Ristriani, Tutik; Stier, Gunter; Masson, Murielle; Cavusoglu, Nukhet; Van Dorsselaer, Alain; Weiss, Etienne; Kieffer, Bruno; Trave, Gilles
CS Laboratoire d'Immunotechnologie UMR CNRS 7100, Ecole Supérieure de Biotechnologie de Strasbourg, Illkirch, 67400, Fr.
SO Biochemistry (2003), 42(17), 4909-4917
CODEN: BICHAW; ISSN: 0006-2960
PB American Chemical Society
DT Journal
LA English
AB E6 is a viral oncoprotein implicated in cervical cancers, produced by high-risk human **papillomaviruses** (HPVs). Structural data concerning this protein are scarce due to the difficulty of producing recombinant E6. Recently, we described the expression and purifn. of a stable, folded, and biol. active HPV16 E6 mutant called E6 6C/6S. Here, we analyzed the domain substructure of this mutated E6. Nonspecific proteolysis of full-length E6 6C/6S (158 residues) yielded N-terminal and C-terminal fragments encompassing residues 7-83 and 87-158, resp. The C-terminal fragment of residues 87-158 was cloned, overexpressed, and purified at concns. as high as 1 mM. The purified domain retains the selective four-way DNA junction recognition activity of the full-length E6 protein. Using UV absorption, UV fluorescence, CD, and **NMR**, we show that the peptide is primarily monomeric and folded with equal proportions of .alpha.-helix and .beta.-sheet secondary structure.
RE.CNT 62 THERE ARE 62 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 2 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
AN 2001:781257 CAPLUS
DN 135:315579
TI Nuclear magnetic resonance methods for identifying sites in **papillomavirus** E2 protein
IN Stockman, Brian J.
PA Pharmacia + Upjohn Company, USA
SO PCT Int. Appl., 29 pp.
CODEN: PIXXD2
DT Patent
LA English
FAN.CNT 1

not examined anywhere

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
WO 2001079852	A2	20011025	WO 2001-US11621	20010410
WO 2001079852	A3	20020613		

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM
RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF,

BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG

US 2001051333 A1 20011213 US 2001-829872 20010410

PRAI US 2000-197459P P 20000417

US 2000-211055P P 20000613

US 2001-268444P P 20010213

US 2001-829872 A 20010410

AB **NMR** methods for identifying sites in a **DNA-binding** and dimerization domain of a **papillomavirus E2** protein are disclosed. Preferably the sites are ligand binding sites.

L2 ANSWER 3 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2001:20460 CAPLUS

DN 134:203956

TI DNA tightens the dimeric **DNA-binding** domain of human **papillomavirus E2** protein without changes in volume

AU Lima, Luis Mauricio T. R.; Foguel, Debora; Silva, Jerson L.

CS Departamento de Medicamentos, Faculdade de Farmacia, Universidade Federal do Rio de Janeiro, Rio de Janeiro, 21941-590, Brazil

SO Proceedings of the National Academy of Sciences of the United States of America (2000), 97(26), 14289-14294

CODEN: PNASA6; ISSN: 0027-8424

PB National Academy of Sciences

DT Journal

LA English

AB The recognition of palindromic specific DNA sequences by the human **papillomavirus (HPV) E2** proteins is responsible for regulation of virus transcription. The dimeric **E2 DNA-binding** domain of HPV-16 (E2c) disassociates into a partially folded state under high hydrostatic pressure. We show here that pressure-induced monomers of E2c are highly structured, as evidenced by **NMR** hydrogen-deuterium exchange measurements. On binding to both specific and nonspecific DNA, E2c becomes stable against pressure. Competitive binding studies using fluorescence polarization of fluorescein-labeled DNA demonstrate the reversibility of the specific binding. To assess the thermodynamic parameters for the linkage between protein dissociation and **DNA binding**, urea denaturation curves were obtained at different pressures in the presence of specific and nonspecific DNA sequences. The change in free energy on denaturation fell linearly with increase in pressure for both protein-DNA complexes, and the measured volume change was similar to that obtained for E2c alone. The data show that the free energy of dissociation increases when E2c binds to a nonspecific DNA sequence but increases even more when the protein binds to the specific DNA sequence. Thus, specific complexes are tighter but do not entail variation in the volume change. The thermodynamic data indicate that DNA-bound E2c disassociates into monomers bound to DNA. The existence of monomeric units of E2c bound to DNA may have implications for the formation of DNA loops, as an additional target for viral and host factors binding to the loosely associated dimer of the N-terminal module of the E2 protein.

RE.CNT 49 THERE ARE 49 CITED REFERENCES AVAILABLE FOR THIS RECORD

ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 4 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2000:315669 CAPLUS

DN 133:27862

TI Chemical Shift Mapped **DNA-Binding** Sites and ¹⁵N Relaxation Analysis of the C-Terminal KH Domain of Heterogeneous Nuclear Ribonucleoprotein K

AU Baber, James L.; Levens, David; Libutti, Daniel; Tjandra, Nico

CS Laboratory of Biophysical Chemistry, National Heart Lung and Blood Institute National Institutes of Health, Bethesda, MD, 20892, USA

SO Biochemistry (2000), 39(20), 6022-6032

CODEN: BICHAW; ISSN: 0006-2960

PB American Chemical Society

DT Journal

LA English

AB The K homol. (KH) motif is one of the major classes of nucleic acid binding proteins. Some members of this family have been shown to interact with DNA while others have RNA targets. There have been no reports contg. direct exptl. evidence regarding the nature of KH module-DNA interaction. In this study, the interaction of the C-terminal KH domain of heterogeneous nuclear ribonucleoprotein K (KH3) with it's cognate single-stranded DNA (ssDNA) are investigated. Chem. shift perturbation mapping indicates that the first two helices, the conserved GxxG loop, .beta.1, and .beta.2, are the primary regions involved in **DNA binding** for KH3. The nature of the KH3-ssDNA interaction is further illuminated by a comparison of backbone 15N relaxation data for the bound and unbound KH3. Relaxation data are also used to confirm that the backbone of wild-type KH3 is structurally identical to that of the G26R mutant KH3, which was previously published. Amide proton exchange expts. indicate that the two helices involved in **DNA binding** are less stable than other regions of secondary structure and that a large portion of KH3 backbone amide hydrogens are protected in some manner upon ssDNA binding. The major backbone dynamics features of KH3 are similar to those of the structurally comparable human **papillomavirus-31 E2 DNA binding** domain. Secondary structure information for ssDNA-bound wild-type KH3 is also presented and shows that binding results in no global changes in the protein fold.

RE.CNT 74 THERE ARE 74 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 5 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN

AN 2000:277203 CAPLUS

DN 133:39626

TI Folding of a dimeric .beta.-barrel: residual structure in the urea denatured state of the human **papillomavirus E2 DNA binding** domain

AU Mok, Yu-Keung; Alonso, Leonardo G.; Lima, Mauricio T. R.; Bycroft, Mark; De Prat-Gay, Gonzalo

CS MRC Unit for Protein Function and Design, Cambridge University Chemical Laboratory, Cambridge, CB2 1EW, UK

SO Protein Science (2000), 9(4), 799-811

CODEN: PRCIEI; ISSN: 0961-8368

PB Cambridge University Press

DT Journal

LA English

AB The dimeric .beta.-barrel is a characteristic topol. initially found in the transcriptional regulatory domain of the E2 **DNA binding** domain from **papillomaviruses**. We have previously described the kinetic folding mechanism of the human HPV-16 domain, and, as part of these studies, we present a structural characterization of the urea-denatured state of the protein. We have obtained a set of chem. shift assignments for the C-terminal domain in urea using heteronuclear **NMR** methods and found regions with persistent residual structure. Based on chem. shift deviations from random coil values, ³JNHN.alpha. coupling consts., heteronuclear single quantum coherence peak intensities, and nuclear Overhauser effect data, we have detd. clusters of residual structure in regions corresponding to the **DNA binding** helix and the second .beta.-strand in the folded conformation. Most of the structures found are of non-native nature, including turn-like conformations. Urea denaturation at equil. displayed a loss in protein concn. dependence, in abs. parallel to a similar deviation obsd. in the folding rate const. from kinetic expts. These results strongly suggest an alternative folding pathway in which a dimeric intermediate is formed and the rate-limiting step becomes first order at high protein concns. The structural elements found in the denatured state would collide to yield productive interactions, establishing an intermol. folding nucleus at high protein concns. We

discuss our results in terms of the folding mechanism of this particular topol. in an attempt to contribute to a better understanding of the folding of dimers in general and intertwined dimeric proteins such as transcription factors in particular.

RE.CNT 51 THERE ARE 51 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 6 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1999:720361 CAPLUS
DN 132:46491
TI Structural Correlates for Enhanced Stability in the E2 **DNA-Binding** Domain from Bovine **Papillomavirus**
AU Veeraraghavan, Sudha; Mello, Cecilia C.; Androphy, Elliot J.; Baleja, James D.
CS Department of Biochemistry, Tufts University School of Medicine, Boston, MA, 02111, USA
SO Biochemistry (1999), 38(49), 16115-16124
CODEN: BICHAW; ISSN: 0006-2960
PB American Chemical Society
DT Journal
LA English
AB Papillomaviral E2 proteins participate in viral DNA replication and transcriptional regulation. We have solved the soln. structure of the **DNA-binding** domain of the E2 protein from bovine **papillomavirus** (BPV-1). The structure calcn. used 2222 distance and 158 dihedral angle restraints for the homodimer (202 residues in total), which were derived from homonuclear and heteronuclear multidimensional **NMR** (NMR) spectroscopic data. The root-mean-square deviation for structured regions of the monomer when superimposed to the av. is 0.73 +/- 0.10 .ANG. for backbone atoms and 1.42 +/- 0.16 .ANG. for heavy atoms. The 101 residue construct used in this study (residues 310-410) is about 4.5 kcal/mol more stable than a minimal domain comprising the C-terminal 85 amino acid residues (residues 326-410). The structure of the core domain contained within BPV-1 E2 is similar to the corresponding regions of other papillomaviral E2 proteins. Here, however, the extra N-terminal 16 residues form a flap that covers a cavity at the dimer interface and play a role in **DNA binding**. Interactions between residues in the N-terminal extension and the core domain correlate with the greater stability of the longer form of the protein relative to the minimal domain.

RE.CNT 44 THERE ARE 44 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 7 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1999:376250 CAPLUS
DN 131:196003
TI High Precision Solution Structure of the C-terminal KH Domain of Heterogeneous Nuclear Ribonucleoprotein K, a c- myc Transcription Factor
AU Baber, James L.; Libutti, Daniel; Levens, David; Tjandra, Nico
CS Laboratory of Biophysical Chemistry, National Institutes of Health, Bethesda, MD, 20892-0380, USA
SO Journal of Molecular Biology (1999), 289(4), 949-962
CODEN: JMOBAK; ISSN: 0022-2836
PB Academic Press
DT Journal
LA English
AB Among it's many reported functions, heterogeneous nuclear ribonucleoprotein (hnRNP) K is a transcription factor for the c-myc gene, a proto-oncogene crit. for the regulation of cell growth and differentiation. We have detd. the soln. structure of the Gly26 Arg mutant of the C-terminal K-homol. (KH) domain of hnRNP K by **NMR** spectroscopy. This is the first structure investigation of hnRNP K. Backbone residual dipolar couplings, which provide information that is fundamentally different from the std. NOE-derived distance restraints,

were employed to improve structure quality. An independent assessment of structure quality was achieved by comparing the backbone ^{15}N T1/T2 ratios to the calcd. structures. The C-terminal KH module of hnRNP K (KH3) is revealed to be a three-stranded β -sheet stacked against three α -helices, two of which are nearly parallel to the strands of the β -sheet. The Gly26 Arg mutation abolishes single-stranded **DNA binding** without altering the overall fold of the protein. This provides a clue to possible nucleotide binding sites of KH3. It appears unlikely that the solvent-exposed side of the β -sheet will be the site of protein-nucleic acid complex formation. This is in contrast to the earlier theme for protein-RNA complexes incorporating proteins structurally similar to KH3. We propose that the surface of KH3 that interacts with nucleic acid is comparable to the region of DNA interaction for the double-stranded **DNA-binding** domain of bovine **papillomavirus-1 E2** that has a three-dimensional fold similar to that of KH3. (c) 1999 Academic Press.

RE.CNT 82 THERE ARE 82 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 8 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1998:470033 CAPLUS
DN 129:199516
TI ^1H , ^{15}N , and ^{13}C NMR resonance assignments for the **DNA-binding** domain of the BPV-1 E2 protein
AU Veeraraghavan, Sudha; Mello, Cecilia C.; Lee, Karen M.; Androphy, Elliot J.; Baleja, James D.
CS Department of Biochemistry, Tufts University School of Medicine, Boston, MA, 02111, USA
SO Journal of Biomolecular NMR (1998), 11(4), 457-458
CODEN: JBNME9; ISSN: 0925-2738
PB Kluwer Academic Publishers
DT Journal
LA English
AB NMR expts. were run on bovine **papillomavirus** BPV-1 E2 protein which was unlabeled or labeled with ^{15}N or ^{13}C . Backbone, side-chain proton, and chem. shift assignments were obtained.

RE.CNT 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD
ALL CITATIONS AVAILABLE IN THE RE FORMAT

L2 ANSWER 9 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN
AN 1997:603441 CAPLUS
DN 127:229222
TI NMR-Based Discovery of Lead Inhibitors That Block **DNA Binding** of the Human **Papillomavirus** E2 Protein
AU Hajduk, Philip J.; Dinges, Jurgen; Miknis, Gregory F.; Merlock, Megan; Middleton, Tim; Kempf, Dale J.; Egan, David A.; Walter, Karl A.; Robins, Terry S.; Shuker, Suzy B.; Holzman, Thomas F.; Fesik, Stephen W.
CS Pharmaceutical Products Division, Abbott Laboratories, Abbott Park, IL, 60064-3500, USA
SO Journal of Medicinal Chemistry (1997), 40(20), 3144-3150
CODEN: JMCMAR; ISSN: 0022-2623
PB American Chemical Society
DT Journal
LA English
AB The E2 protein is required for the replication of human **papillomaviruses** (HPVs), which are responsible for anogenital warts and cervical carcinomas. Using an NMR-based screen, we tested compds. for binding to the **DNA-binding** domain of the HPV-E2 protein. Three classes of compds. were identified which bound to two distinct sites on the protein. Biphenyl and biphenyl ether compds. contg. a carboxylic acid bind to a site near the DNA recognition helix and inhibit the binding of E2 to DNA. Benzophenone-contg. compds. which lack a carboxylic acid group bind to the β -barrel formed by the dimer interface and exhibit negligible effects on the binding of E2 to

DNA. Structure-activity relationships from the biphenyl and biphenyl ether compds. were combined to produce a compd. [5-(3'-(3'',5''-dichlorophenoxy)phenyl)-2,4-pentadienoic acid] with an IC50 value of approx. 10 .mu.M. This compd. represents a useful lead for the development of antiviral agents that interfere with HPV replication and further illustrates the usefulness of the SAR by NMR method in the drug discovery process.

L2 ANSWER 10 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1996:79340 CAPLUS

DN 124:110297

TI Solution Structure of the **DNA-Binding** Domain of a Human **Papillomavirus** E2 Protein: Evidence for Flexible **DNA-Binding** Regions

AU Liang, Heng; Petros, Andrew M.; Meadows, Robert P.; Yoon, Ho Sup; Egan, David A.; Walter, Karl; Holzman, Thomas F.; Robins, Terry; Fesik, Stephen W.

CS Pharmaceutical Discovery Division, Abbott Laboratories, Abbott Park, IL, 60064, USA

SO Biochemistry (1996), 35(7), 2095-103

CODEN: BICHAW; ISSN: 0006-2960

PB American Chemical Society

DT Journal

LA English

AB The three-dimensional structure of the **DNA-binding** domain of the E2 protein from human **papillomavirus**-31 was detd. by using multidimensional heteronuclear **NMR** spectroscopy. A total of 1429 **NMR**-derived distance and dihedral angle restraints were obtained for each of the 83-residue subunits of this sym. dimer. The av. root mean square deviations of 20 structures calcd. using a distance geometry-simulated annealing protocol are 0.59 and 0.90 .ANG. for the backbone and all heavy atoms, resp., for residues 2-83. The structure of the human virus protein free in soln. consists of an eight-stranded .beta.-barrel and two pairs of .alpha.-helixes. Although the overall fold of the protein is similar to the crystal structure of the bovine **papillomavirus**-1 E2 protein when complexed to DNA, several small but interesting differences were obsd. between these two structures at the subunit interface. In addn., a .beta.-hairpin that contacts DNA in the crystal structure of the protein-DNA complex is disordered in the **NMR** structures, and steady-state 1H-15N heteronuclear NOE measurements indicate that this region is highly mobile in the absence of DNA. The recognition helix also appears to be flexible, as evidenced by fast amide exchange rates. This phenomenon has also been obsd. for a no. of other **DNA-binding** proteins and may constitute a common theme in protein/DNA recognition.

L2 ANSWER 11 OF 11 CAPLUS COPYRIGHT 2003 ACS on STN

AN 1994:551501 CAPLUS

DN 121:151501

TI 1H **NMR** studies of the mercuric ion binding protein MerP: sequential assignment, secondary structure and global fold of oxidized MerP

AU Eriksson, Per Olof; Sahlman, Lena

CS Dep. Phys. Chem., Univ. Umea, Umea, S-901 87, Swed.

SO Journal of Biomolecular NMR (1993), 3(6), 613-26

CODEN: JBNME9; ISSN: 0925-2738

DT Journal

LA English

AB The oxidized form of the mercuric ion binding protein MerP has been studied by two-dimensional **NMR**. In this work, the 1H **NMR** spectrum of oxidized MerP (closed disulfide bridge) has been assigned by using homonuclear 2D **NMR** techniques. The secondary structure and global fold have been inferred from the nuclear Overhauser effect (NOE) data. The secondary structure comprises four .beta.-strands

and two .alpha.-helixes, in the order .beta.1.alpha.1.beta.2.beta.3.alpha.2.beta.4. The protein folds into an antiparallel .beta.-sheet, .beta.2.beta.3.beta.1.beta.4, with the two antiparallel helixes on one side of the sheet. The folding topol. is similar to that of acylphosphatase, the activation domain of porcine pancreatic procarboxypeptidase B, the **DNA-binding** domain of bovine **papillomavirus**-1 E2 and the RNA-binding domains of the U1 snRNP A and hnRNP C proteins. However, there is no structural similarity between MerP and other bacterial periplasmic binding proteins.